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IN-SILICO DOCKING OF CURCUMIN AND ITS DERIVATIVES AGAINST AMYLOID BETA PEPTIDE IN ALZHEIMER'S DISEASE

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ABSTRACT

The accumulation of aggregated β-Amyloid (Aβ) in the brain is a hallmark of Alzheimer's disease and is thought to play a role in the neurotoxicity associated with the disease. The main objective of docking studies, which is one important tool of computational methods in identifying the lead compounds as a potential drug, is accurate structural modeling and correct prediction of activity. In this perspective, curcumin and curcumin derivatives viz. CurL 1-8 were selected for the study. Amlyoid beta (Aβ) peptide was used a target. Lamarckian genetic algorithm methodology was employed for docking simulations using Docking server. The three important parameters like binding energy, inhibition constant and intermolecular energy were determined. The curcumin derivative (CurL6) showed lesser binding energy (-3.26 Kcal/mol) when compared with that of curcumin (-2.62 Kcal/mol). All the derivatives are showing intermolecular energy from -6.89Kcal/ mol to -4.63 Kcal/mol and inhibition constant was found to be 4.10 – 65.06 mM).

Keywords: Alzheimer's disease, Beta amyloid, Curcumin; Molecular docking.

INTRODUCTION

Alzheimer's disease (AD) is a progressive, age-related, neuro-generative disease and cause of dementia [1-3]. Till date, no cure is there for this disease and the situation get worsened as the disease progresses, ultimately causing death. AD is characterized by progressive loss of synapses and neurons, accumulation of amyloid beta peptide (Aβ) and neurofibrillary tangles (NFTs), composed of hyperphosphorylated tau protein [4]. Although the root cause of most

of the Alzheimer's cases is unknown but there are some hypotheses proposed by various researchers trying to explain the cause of this disease. The first hypothesis, also known as cholinergic hypothesis and on which most of the current drugs are based, proposed that reduced synthesis of acetylcholine (a neurotransmitter) is the cause of AD [5]. So, for treating AD main medication used is cholinesterase inhibitor. Donepezil, Rivastigmine and Galantamine are three commonly used cholinesterase inhibitor [6, 7]. These drugs prevent the functioning of acetylcholinesterase which breaks down acetylcholine thus the level of acetylcholine increases in brain leading to increased communication between the nerve cell and thus temporarily stabilize the symptoms of AD. According to second hypothesis *i.e.* Amyloid hypothesis, amyloid plaque deposits in the brain are the main cause of AD. Amyloid beta (Aβ), 4 kDa protein is the main component of amyloid plaque deposits and accumulation of abnormally folded Aβ proteins in the brains leads to AD [8, 9]. Amyloid plaques are nothing but clumps of Aβ which originates from proteolytic cleavage of amyloid precursor protein (APP) by membrane associated β and γ secretase [10]. This process generates various peptides of different size and out of them A β_{40} (1-40 amino acids) and $A\beta_{42}$ (1-42 amino acids) are the most abundant ones [11, 12]. There are two possible pathways for proteolytic cleavage of APP *viz.* amyloidogenic and non-amyloidogenic pathways. In amyloidogenic pathway, β secretase (BACE 1) cleaves APP and as a result s-AAP β (secreted amyloid precursor protein β) and C99 (β-carboxy terminal fragment or β-CTF) are produced. C99 can further be cleaved by γ secretase and give rise to Aβ and AICD (APP intracellular domain). Proteolysis by γ secretase is heterogenous in nature and produces 40 residue peptide Aβ-40 (majority of full length Aβ species) and a 42 residue peptide Aβ-42 (small proportion and carboxy terminal variant) [13,14] ; these molecules tend to aggregate to form oligomers. The longer Aβ-42 is more hydrophobic and much prone to fibril formation than Aβ-40. Thus Aβ-42 is the main Aβ species in cerebral plaques. In non-amyloidogenic pathway [15], APP cleavage is initiated by α secretase and produces s-AAP α (secreted amyloid precursor protein α) and C83 (α -carboxy terminal fragment or α -CTF). C83 can be further cleaved by γ secretase and produces P3 and AICD. Presently, there is no cure for this devastating disease which prompted researchers to look for new drugs to cope with it or which can hinder the disease process. One of the effective ways to treat AD is to inhibit misfolding and reverse aggregation of amyloid peptides. The current lot of drugs available for treating AD includes antioxidant drugs, nonsteroidal anti-inflammatory drugs (NSAID) naproxen and ibuprofen which are potential candidates to control Aβ aggregation. These drugs improve the function of still intact neurons, but do not inhibit the ongoing degenerative process leading to neuronal

cell death. Thus, current pharmaceutical drugs only provide temporarily relief [16, 17]. The available pharmaceutical drugs are not sufficient to address the problem of AD which leads the researchers to look for other alternatives with more efficacy and lesser side effects such as neutraceutical compounds.

Turmeric, widely used as a part of daily diet in Asian countries for long, has not shown any toxicity and is shown to exhibit various therapeutic activities for a wide variety of diseases and conditions. The major active component of turmeric is a yellow compound curcumin, naturally occurring polyphenol, which is a dried powder isolated from rhizomes of *Curcumin longa* [18]. Curcumin is shown to have some biological properties such as antimicrobial, antiviral, antifungal and anti-inflammatory activities [19]. Curcumin is shown to have beneficial effects in diseases of the neurological system including Alzheimer's disease [20].Exhaustive literature survey reveals that curcumin can inhibit Aβ aggregation. There is also well documented proof that curcumin can interfere with Aβ oligomerization better than ibuprofen and naproxen. Experimental evidence suggests that curcumin acts as chelator for metals which are concentrated in the AD brain and bind with the metals on beta amyloid thus potentially reducing amyloid formation. Computer aided drug designing (in silico method) is a crucial component of drug discovery programmes [21-23]. Out of many computational tools available, Molecular docking is the most commonly employed one for calculating binding affinities and predicting binding sites. Docking studies helps in accurate structural modeling and correct prediction of activity. In the present study Molecular docking has been used for the accurate study of binding affinity of curcumin and its derivatives to amyloid peptide (Aβ1-42) so that possibility of using curcumin or its derivatives in treating AD can be explored.

MATERIALS AND METHODS

Selection of amyloid beta peptide structure

Homo sapiens brain's beta amyloid peptide 3D crystal structure was downloaded from the PDB structural database site (http://www.rcsb.org/pdb). The PDB ID of the peptide was 1IYT and used as a docking target.

Ligand preparation

In present study, the ligand was curcumin (Pubchem ID 969516) and its eight derivatives. The chemical structure was drawn and uploaded to the ligands section of

DockingServer.com. The drawn ligands can be saved in MOL format. Once ligand is uploaded, the algorithm automatically computes the 3D structure of the ligand compound. The structures of ligands used in experiment are shown in Figure 1.

Identification of the amyloidogenic regions in the proteins

Amyloidogenic regions in polypeptide chains are very important because such regions are responsible for amyloid formation and aggregation. Amyloidogenic region of the protein was predicted using web server FoldAmyloid, which predicts the specific sequence(s) on a polypeptide chain which are likely to form aggregates based on the physicochemical properties of the amino acid residues

Docking methodology

There is a wide range of software packages available for the conduct of molecular docking simulations like, AutoDock, DOCK, GOLD, FlexX and ICM. Docking calculations were carried out using DockingServer [24]. Chimera was used to visualize the docking complex and to generate the 3D structures of the same Docking simulations were performed using the Lamarckian genetic algorithm (LGA) and the Solis & Wets local search method [25].

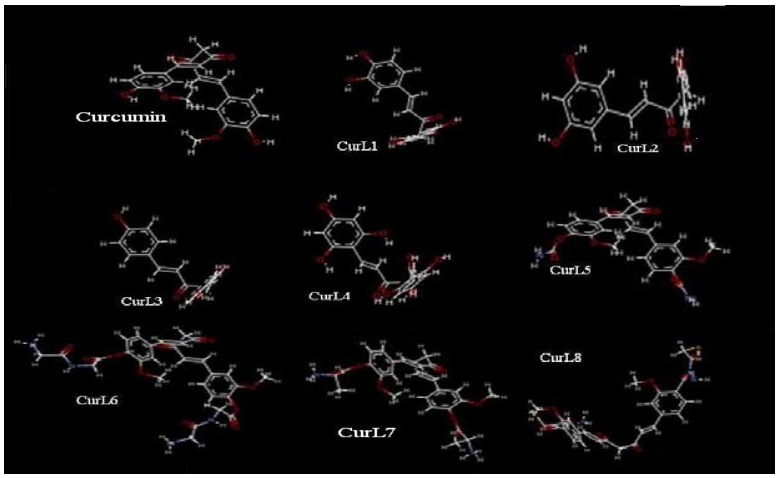

Figure1 3D structures of curcumin and its derivatives.

RESULTS AND DISCUSSION

Structure of amyloid beta (Aβ) and amyloidogenic region

The three dimensional protein structure of Aβ peptide (PDB ID-1IYT) was retrieved from protein data bank. Aβ is a short peptide with 1-42 residues. The structure showed two alpha helical regions which are joined by regular type I beta turn [26]. First helical region is that of residues encompassing residues 8-25 and second encompassing region 28-38. FoldAmyloid

server was developed for prediction of amyloidogenic regions in a protein or peptide [27]. The amyloidic region of the peptide in first helix is amino acid sequence 16-21 and in second helix 32-36 amino acid sequences (Figure 2).

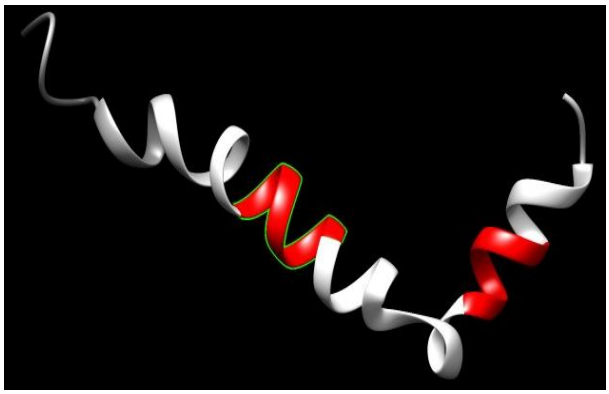

Figure 2. Solution structure of the amyloid beta-peptide. The amyloidogenic residues as predicted by the FoldAmyloid server are highlighted in red color.

Docking analysis

Analysis of the target/ligand complex models generated after successful docking of the curcumin and curcumin based ligand were based on the parameters such as hydrogen bond interactions, binding energy and orientation of the docked compound within the amyloidic region.

Curcumin to beta amyloid

The ligand of choice was Curcumin (PubChem ID 969516) (Fig.1) Docking server computed the free energy of binding to be -2.62 kcal/mol and inhibition constant (Ki) computed was 12.10 mM, while the total energy of binding was -5.10kcal/mol (Table 1). Two hydrogen bonds were seen. Figure 3 show that these two hydrogen bonds were formed with side chains of lysine-16 residue which lie directly in the amyloidogenic stretch KLVFFA. Thus Curcumin directly binds to Amyloidic streach16-21 preventing aggregation.

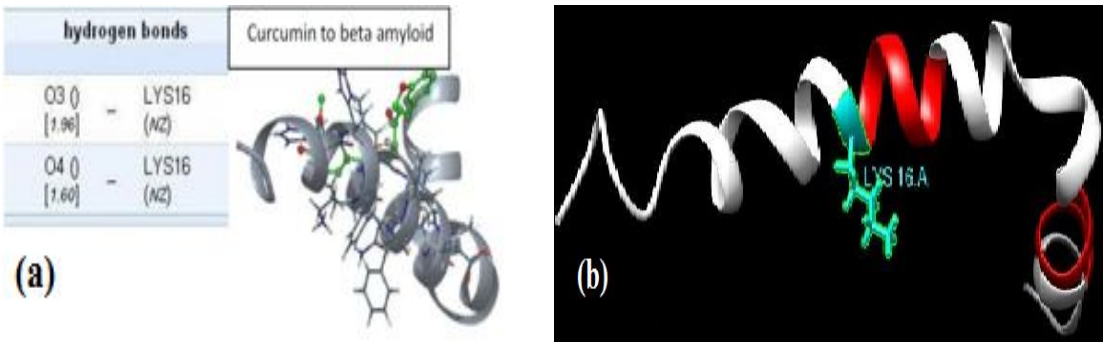

Figure 3 (a) Interaction between Curcumin and beta amyloid obtained from DockingDerver.com (b) corresponding image obtained from chimera software showing residues forming hydrogen bonds highlighted as blue, while Amyloidic regions are shown in red.

Table 1 Docking Scores of Curcumin and its derivatives

Ligand	Free binding energy (kcal/mol)	Inhibition constant mM	Total intermolecular energy (kcal/mol)
Curcumin	-2.62	12.10	-5.10
CurL 1	-2.94	6.99	-4.63
CurL 2	-2.42	16.90	-5.21
CurL 3	-3.24	4.21	-5.54
CurL 4	-1.62	65.06	-4.49
CurL 5	-2.65	11.46	-5.02
CurL 6	- 3.26	4.10	-6.89
CurL 7	- 1.85	44.41	-5.70
CurL 8	-2.13	27.60	-5.49

CurL1 to beta amyloid

Calculations by Dockingserver.com showed that CurL1 displayed free energy of binding of -2.94 kcal/mol and inhibition constant (Ki) computed was 6.99 mM, while the total energy of binding was -4.73kcal/mol (Table1). Three hydrogen bonds were seen. Figure 4 shows that two of these hydrogen bonds were formed with side chain of lysine 16 and one with side chain of alanine 21 residue, thus showing strong interactions with Amyloidogenic stretch KLVFFA which may indeed be efficient in inhibiting amyloidogenesis.

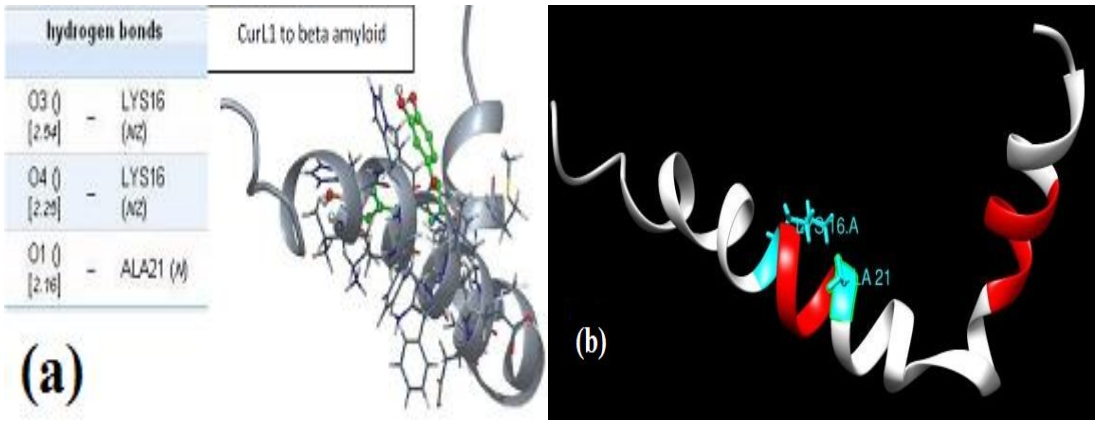

Figure 4 (a) interaction between CurL1 and beta amyloid obtained from DockingDerver.com (b) Corresponding image obtained from chimera software showing residues forming hydrogen bonds highlighted as blue, while Amyloidic regions are shown in red

CurL2 to beta amyloid

CurL2 however showed insufficient free energy of binding -2.42 kcal/mol and inhibition constant (Ki) computed was 16.90 mM, while the total energy of binding was -5.21 kcal/mol (Table 1) Figure 5 displays that no hydrogen bonds were seen or strong side chain interactions were seen. Thus CurL2's has lower efficiency than Curcumin.

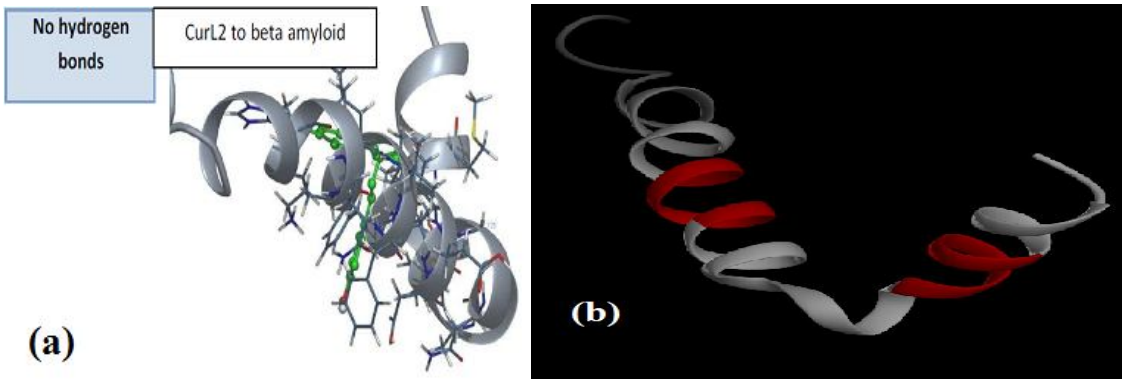

Figure 5 (a) Interaction between CurL2 and beta amyloid obtained from DockingDerver.com (b) Corresponding image obtained from chimera software showing residues forming hydrogen bonds highlighted as blue, while Amyloidic regions are shown in red.

CurL3 to beta amyloid

Dockingserver.com calculations of CurL3 and beta amyloid peptides interaction showed free energy of binding -3.24 kcal/mol and inhibition constant (Ki) 4.21 mM, while the total energy of binding was computed -5.54kcal/mol (Table 1). Two hydrogen bonds were seen (Figure 6). One of these two hydrogen bond is with side chain of lysine 16 residue and one with glycine 25 side chain. Both of these interactions are near amyloidogenic stretch KLVFFA and thus show possible inhibition to amyloidogenesis.

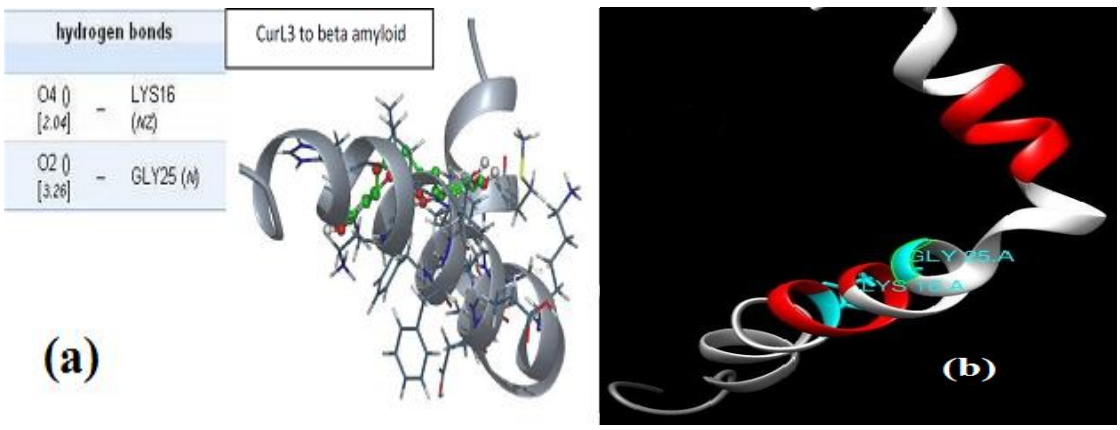

Figure 6 (a) Interaction between CurL3 and beta amyloid obtained from DockingDerver.com (b) corresponding image obtained from chimera software showing residues forming hydrogen bonds highlighted as blue, while Amyloidic regions are shown in red.

CurL4 to beta amyloid

Calculations for interaction between CurL4 and beta amyloid showed Free energy of binding -1.62 kcal/mol and inhibition constant (Ki) 65.06 mM, while the total energy of binding was -4.49kcal/mol (Table 1). Three hydrogen bonds were seen shows that like CurL1 two of these hydrogen bonds are with side chain of lysine 16 and one with side chain of alanine (Figure 7). However the binding energy was highest and thus least efficient among the ligands studied.

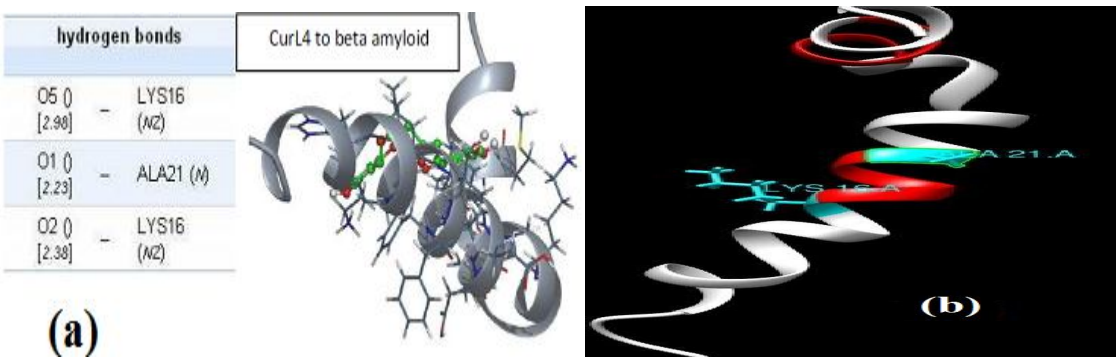

Figure 7 (a) Interaction between CurL4 and beta amyloid obtained from DockingDerver.com (b) Corresponding image obtained from chimera software showing residues forming hydrogen bonds highlighted as blue, while Amyloidic regions are shown in red.

CurL5 to beta amyloid

For CurL5 Docking server.com showed free energy of binding -2.65 kcal/mol and inhibition constant (Ki) computed was 11.46 mM, while the total energy of binding was calculated to be -5.02kcal/mol (Table 1). Six hydrogen bonds were seen. Figure 8 shows two of these interactions are with side chains of Histidine 13, one with side chain of Histidine 14, two with side chain of Lysine 16 and one with side chain of Alanine 21 thus showing strong interaction with amyloidogenic stretch KLVFFA and possible inhibition of oligomer and fibrilisation.

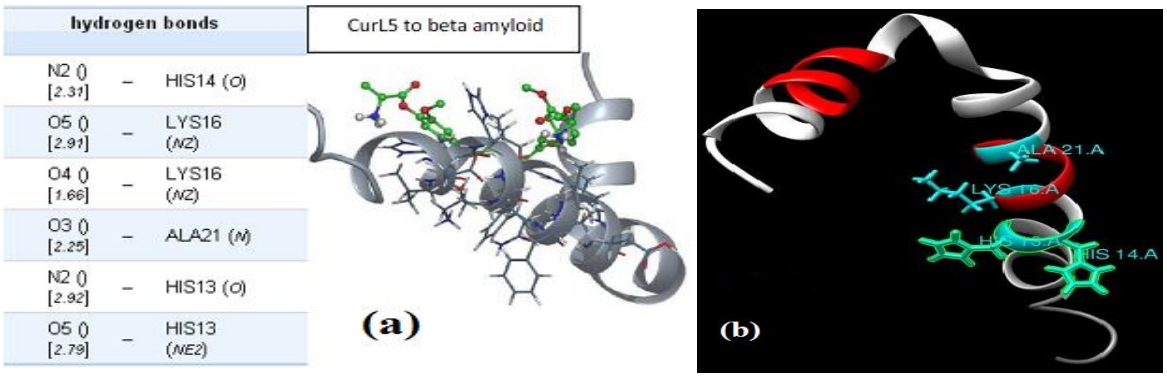

Figure 8 (a) Interaction between CurL5 and beta amyloid obtained from DockingDerver.com (b) Corresponding image obtained from chimera software showing residues forming hydrogen bonds highlighted as blue, while Amyloidic regions are shown in red.

CurL6 to beta amyloid

Dockingserver.com calculations of CurL6 and beta amyloid peptides interaction showed Free energy of binding -3.26 kcal/mol and inhibition constant (Ki) computed was 4.10 mM, while the total energy of binding was -6.89 kcal/mol (Table 1). Four hydrogen bonds were seen (Figure 9). Three of these hydrogen bonds were with side chains of lysine 16 residue and one with side chain of Alanine 21. The low binding energy and strong interaction with KLVFFA amyloidogenic stretch make it one of the strongest drug candidate.

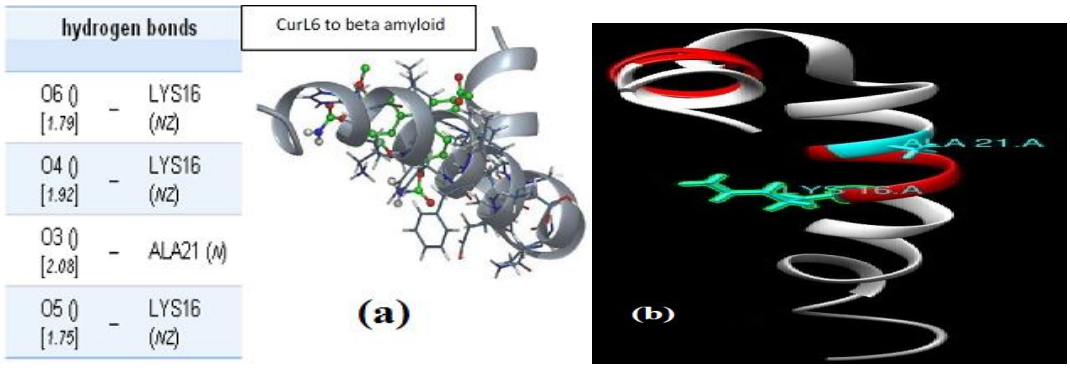

Figure 9 (a) Interaction between CurL6 and beta amyloid obtained from DockingDerver.com (b) Corresponding image obtained from chimera software showing residues forming hydrogen bonds highlighted as blue, while Amyloidic regions are shown in red.

CurL7 to beta amyloid

Calculations for CurL7 showed Free energy of binding -1.85 kcal/mol and inhibition constant (Ki) computed was 44.41 mM, while the total energy of binding was -5.70 kcal/mol (Table 1). Four hydrogen bonds were seen. Figure 10 shows two of the hydrogen bonds are with Lysine 16 side chain, one with side chain of Leucine 17 and one with side chain of valine 18.

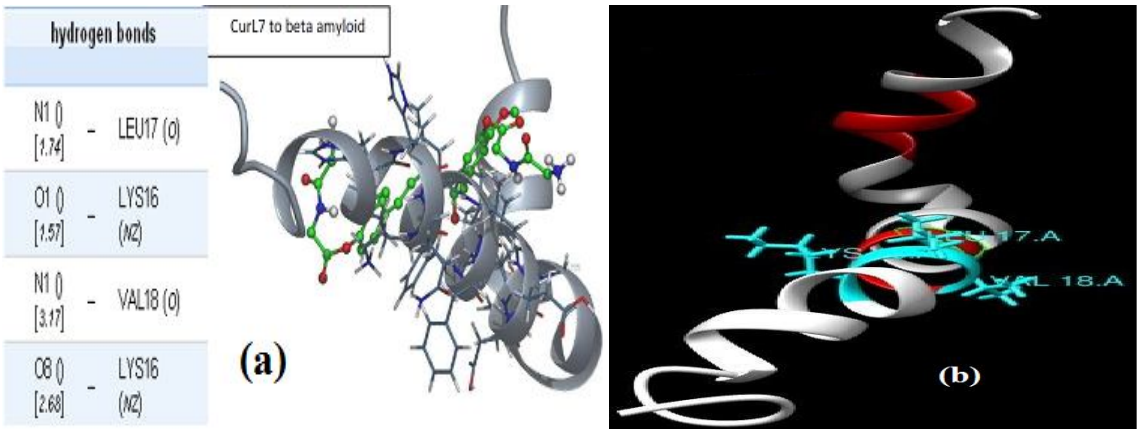

Figure 10 (a) Interaction between CurL7 and beta amyloid obtained from DockingDerver.com (b) Corresponding image obtained from chimera software showing residues forming hydrogen bonds highlighted as blue, while Amyloidic regions are shown in red.

CurL8 to beta amyloid

For CurL8 DockingServer.com showed Free energy of binding -2.13 kcal/mol and inhibition constant (Ki) computed was 27.60 mM, while the total energy of binding was -5.49 kcal/mol (Table 1) Eight hydrogen bonds were seen. Three of the hydrogen bonds were with the side chains of Histidine 13, two with side chains of Histidine 14 and three with the side chains of Lysine 16 residues (Figure 11). All of the ligand interactions were with amyloidogenic stretch KLVFFA.

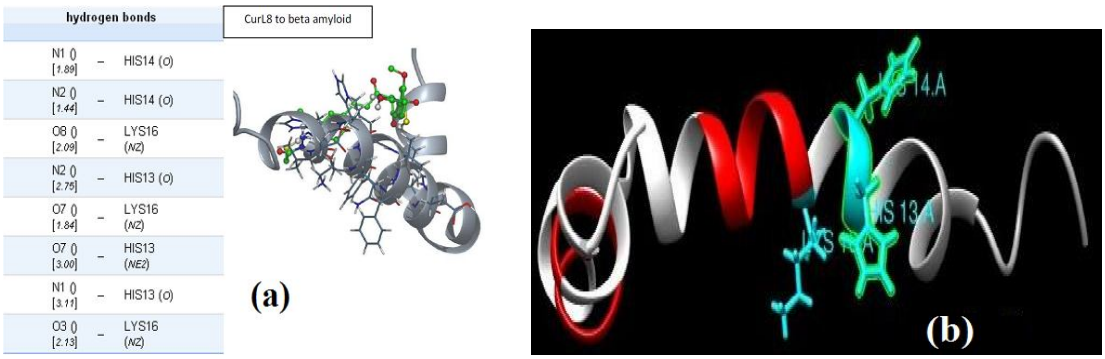

Figure 11 (a) Interaction between CurL8 and beta amyloid obtained from DockingDerver.com (b) corresponding image obtained from chimera software showing residues forming hydrogen bonds highlighted as blue, while Amyloidic regions are shown in red.

CONCLUSION

Small molecules can be used efficiently to bind to beta amyloid peptide and thus inhibit the formation of cytotoxic aggregates. Curcumin is a known ligand for beta amyloid peptide. In the present study various derivatives of Curcumin were virtually interacted with peptide taking curcumin as standard and it was that derived ligands having binding energy and inhibition constant lower then curcumin are possible drugs contenders. Since CurL6 had least binding energy and inhibition constant i.e. -3.26 kcal/mol and 4.10 mM respectively, it is the strongest drug contender for AD. The mode of action for drug would be to inhibit oligomerisation and aggregation of beta amyloid peptide to prevent formation cytotoxic chemical species. Molecular modeling and molecular docking methods still have a long way to run before producing completely reliable results. This could be achieved by increasing the number of parameters considered and using new scoring functions both of which require the use of advanced computational capabilities. Investigations on various associated biochemical compounds such as tau protein, in vivo studies and furthermore clinical trials of the same are required before development of potential chemical entities for the prevention and treatment of AD.

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